Adv. Hort. Sci., 2018 32(3): 389-398 DOI: 10.13128/ahs-23229

AHS
Advances in Horticultural Science

Postharvest control of *Aspergillus niger* in mangos by means of essential oil

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Key words: *Aspergillus niger*, essential oil, mango, postharvest.

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Citation:
JAVADPOUR S., GOLESTANI A., RASTEGAR S., DASTJER M.M., 2018 - *Postharvest control of Aspergillus niger in mangos by means of essential oil*. - Adv. Hort. Sci., 32(3): 389-398

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 14 May 2018
Accepted for publication 12 September 2018

Abstract: The use of essential oil as an alternative mean to synthetic fungicides has been considered in the past years for management of the postharvest decay of fruits in order to ensure more safe and long storage life of these perishable commodities. *Aspergillus niger* is one of the most dangerous fungal pathogen which can cause postharvest diseases in fresh mangos. The aim of this study was to assess the effectiveness of essential oil from four aromatic plants (*Thymus vulgaris*, *Salvia mirzayanii*, *Artemisa persica*, and *Rosmarinus officinalis*) in comparison to fungicide 'Mancozeb' against *A. niger* under *in vitro* and *in vivo* conditions. After inoculation of mango fruits with an isolate of *A. niger* followed by curative treatments with essential oil, the main physical and chemical attributes of mangoes were determined under postharvest condition. The *in vitro* results showed that colonies of *A. niger* were totally inhibited by application of essential oil of *T. vulgaris* (at all the tested concentrations) and *A. persica* (1500 µl/l). While, *S. mirzayanii* showed the lowest effect at 1000 µl/l if compared with the other essential oils. The results of the *in vivo* experiments showed that treatments with *T. vulgaris* and *S. mirzayanii* essential oil had significant (P<0.05) effects in preventing fruit decay at 1000 µl/l after 10 days of storage, while, *R. officinalis* essential oil significantly (P<0.05) reduced deterioration of mango fruits at 500 µl/l, followed by *A. persica*. Rosemary also showed the highest fruit firmness in comparison with other treatments. Also, the essential oils maintained higher chlorophyll content. The results of this work showed that application of essential oil on mangos assurance both a significant preservation on their quality attributes by controlling, at the same time, decaying caused by *A. niger* during the postharvest phase.

1. Introduction

Postharvest diseases are among the major causes of losses of mangos (*Mangifera indica* L.) fresh produce throughout the supply chain. The incidence of the postharvest diseases can also affect the quality of mangos limiting their shelf life up to 3-4 days. In literature is reported that about 17-37% of fresh mangos is wasted after harvesting and marketing (Madan

and Ullosa, 1993). Sharma *et al*. (1994) have reported that about 17.7% of this fresh produce is lost during the storage and marketing. Mango decay caused by the plant pathogenic fungus *Aspergillus niger* is one of the most dangerous postharvest diseases, leading to the losses of fruit quality during storage (Duamkhanmanee, 2008).

It well known from published reports as more negative effects associated to use of chemical fungicides for controlling postharvest diseases have been reported on the human's health and environment (Wightwick *et al.*, 2010). Furthermore, consumers believe that fruits not treated (or minimally-treated) with fungicides are safer for fresh consumption (Du Plooy *et al*., 2009). In the past 20 years there has been a great interest in using essential oils (EOs) to control postharvest diseases, such increasing shelf life of stored fruits (Tripathi and Dubey, 2004). Several studies have also reported on the antifungal activity of *Thymus vulgaris* against different strains of *Colletotrichum gloeosporioides*, *Rhizopus stolonifer*, *Penicillium digitatum* (Abdolahi *et al*., 2010; Sellamuthu *et al.*, 2013), *Aspergillus flavus*, *Aspergillus niger, Aspergillus fumigatus, and Alternaria alternata* (Kumar *et al.*, 2008). Different species of *Salvia* are yet used as antimicrobial agents (Fiore *et al.*, 2006; Kelen and Tepe, 2008), however *Salvia mirzayanii* is an endemic plant which grows only in some parts of Iran, and thus there is no an exhaustive information regarding its effect on *A. niger* (Rechinger, 1986*).* Centeno *et al.* (2010) have reported that extracts from *Rosmarinus officinalis* and *T. vulgaris* could have a significant effect on the control of fungal decaying. Previous studies have also confirmed the interesting antimicrobial activity of *R. officinalis* EO against spoilage and pathogenic food-related fungi (Abdolahi *et al.*, 2010). De Sousa *et al.* (2013), for instance, detected the strong effect of the *Origanum vulgare* and *R. officinalis* EOs in controlling *A. flavus*.

On the other hand, fewer papers report issues regarding to postharvest control of *A. niger* on mango fruits using EOs as an alternative mean to synthetic fungicides. This study was focused to evaluate the effectiveness of EOs derived from four aromatic plants (*T. vulgaris*, *S. mirzayanii, Artemisa persica* and *R. officinalis*) to *in vitro* and *in vivo* suppress the growth of one pathogenic strain of *A. niger* by preserving the mango fruit quality attributes under postharvest condition.

2. Materials and Methods

Plant material and extraction of essential oil

S. mirzayanii and *A. persica* samples were collected from Lar region of Fars Province, Iran (Lat. 27°41' 3'' N and Long. 54°2' 10''E). A *T. vulgaris* sample was collected from Geno region of Hormozgan Province, Iran (Lat. 25° 38' 37.9'' N and Long. 57° 46' 28'' E) and *R. officinalis* samples from Kerman Province, Iran (Lat. 30° 17' 2.1'' N and Long. 57° 5' 0.1'' E). Samples were harvested in vegetative stage (before flowering). The leaves of samples were cut into small pieces and shade-dried at room temperature. The material was then ground to fine powder. The 80 g of plant material were subjected to extraction of EOs by hydro-distillation method for 6 h using a Clevenger's apparatus (Moghaddam *et al*., 2011). The EOs were separately collected, dehydrated using sodium sulphate (Na_2SO_4), and finally stored in a dark bottle at 4°C until tested.

In vitro experiments

Fungi were isolated from mango and their identity was confirmed

The *Aspergillus niger* (PTCC 5010) was supplied by Iranian research organization for science and technology (IROST). Culture of the pathogen organism was maintained on potato dextrose agar (PDA) medium. Stock cultures were grown at 25°C for 7 days to allow for sufficient sporulation. Antifungal effects of EOs were carried out by the Solution Method (SM) according to Pitarokili *et al.* (1999). Inhibitory effects of EO extracted from *S. mirzayanii, A. persica, R. officinalis* and *T. vulgaris* were determined by *in vitro* antifungal assays. To measure the direct fungal inhibition of each EO on mycelial growth of *A. niger*, three different concentrations of them (1000, 1200 and 1500 µl/l) were added to potato dextrose agar (PDA; provided by Scharlau) media before solidification into Petri dishes (8 cm diameter) at 45-50°C. Fungal disks with 5 mm diameter were placed on the middle of Petri dishes and incubated at 25°C for 10 days. Three replicates per each treatment (4 EO × 3 concentrations) including control plate without EO were prepared. Inhibition percentage was determined at the end of incubation time by the index:

$$IP = (dc-dt)/dc \times 100$$

IP = inhibition percentage, dc = mycelium diameter in the control plate, and dt = in the EOs-treated plate.

In vivo experiments

Mangos cv. Halily were harvested from Minab (Lat. 27°07'51'' N and Long. 57°05'13'' E) at the maturity stage of development. Fruit surface was before disinfected with 2% sodium hypochlorite for 3 min, and then artificial inoculations were done by puncturing fruit surface (4 mm deep and 2 mm wide for each inoculation point) with a sterile needle on two sides of each fruit with 40 µl of a conidial suspension containing 10^6 UFC/ml of *A. niger* that it has been sprayed above each wound. After one-day of incubation at 25°C to allow conidia germination into fruit tissue, fruits were treated with 500 and 1000 µl/l EO of each plant in comparison to 0.5 and 1.0 mg/l mancozed. After the treatment (curative), all fruit trials, including the control (fruits were inoculated with conidial suspension without the treatment with essential oils), were placed into boxes and kept at 25°C for 1, 2, and 3 weeks. Decay percentage of fruit was calculated as the number of decayed fruit/ total number of fruit at each replication* 100 (El-Anany *et al.*, 2009).

Physical-chemical analysis

Firmness of each fruit was measured at two points of the equatorial region by using a texture analyzer with a 5 mm probe (Lurton, Taiwan) with units expressed in kg/cm^2. Surface color was measured on each fruit at two opposite sides using a chromameter (CR 400, Minolta) which provided CIE L*, a*, and b* values. L* is color lightness (0= black and 100= white). The a* scale shows in the maximum the red (+a*) and in the minimum the green color (-a*) while the b* ranged from yellow (+b*) to blue (-b*).

The content of ascorbic acid (AA) expressed as mg/100 g fruit weight was determined as described by Molla *et al*. (2011). Aliquots of 10 ml of each sample was homogenized in 100 ml of extraction buffer containing 3% metaphosphoric acid. Aliquots of 10 mL of homogenate was titrated against standard dye 2,6-diclorophenol indophenols to a faint pink color. The method proposed by Lichtenthaler (1987) was used to determine the total chlorophyll and carotenoids content of fruit. The 'Total Soluble Solids' (TSS) content was determined at 20°C using a digital refractometer, and expressed as °Brix. The pH of fruit juice was measured using a Jenway 3320 pH meter calibrated by pH 4 and 7 buffer solutions. The 'Titratable Acidity' (TA) was determined by titration of 5 mL extract with 0.1 mol L^{-1} sodium hydroxide at pH 8.1 and expressed as percent citric acid (Molla *et al.*, 2011). Weight loss, fruit firmness, surface color change, content of AA, total chlorophyll, and carotenoids, and TSS, TA and pH were determined after 1, 2, and 3 weeks of storage at 25°C. These characteristics was done with 3 replicates (3 large fruits for 1 replicate).

Statistical analysis

The experiment was conducted in a randomized factorial designed whit essential oils treatment and storage time as the two factors. Data were submitted to one-way analysis of variance (ANOVA) using SAS version 16.0 and means were separated by the Duncan test at $P<0.05$ (n=3).

3. Results and Discussion

Aspergillus niger mycelia inhibition

In vitro experiments showed that mycelia growth of *A. niger* was significantly suppressed (P<0.05) when treated with the different concentrations of each EO (Fig. 1). The fungal growth was totally inhibited (IP= 100%) by all the concentrations of *T. vulgaris* EO and with 1500 µl/l *A. persica* EO. On the other hand, *S. mirzayanii* EO showed lower effect (IP= 72%) than other EOs when tested at 1000 µl/l concentration.

Our findings are in agreement to the ones described by Kohiyama *et al.* (2015) who reported that *T. vulgaris* EO was able to control the growth of *A. flavus*. Similar observations on the prevention of

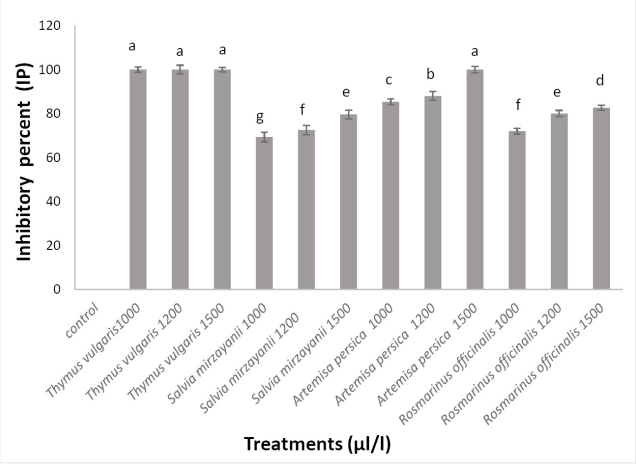

Fig. 1 - Inhibitory effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 1000, 1200 and 1500 µl/l on mycelia growth of *Aspergillus niger* cultures incubated at 25°C for 10 days. Bars indicate the SD of the mean. Different letters indicate significant differences in mean values (p<0.05).

different pathogenic fungi by using EOs have been reported in the previous studies, such as those conducted by Tripathi and Dubey (2004) and Pawar and Thaker (2006). Boubaker *et al.* (2016) reported the antifungal activity of four *Thymus* species EOs against *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum citriaurantii*. Pawar and Thaker (2006) showed that *Cinnamomum zeylanicum, Cinnamomum zeylanicum, Cinnamomum cassia, Cymbopogon citratus* and *Syzygium aromaticum* were the best plant sources for EOs extraction showing a noticeable inhibitory effect against *A. niger.* It was also reported that the mycelial growth of *A. niger* was inhibited by application of 2.5 and 3.0 µg/ml of *Citrus sinensis* oil (sweet orange) in Potato Dextrose Broth and Agar Water medium, respectively (Sharma and Tripathi, 2008).

Modifications on fungal structures induced by the EOs afore-quoted might be due to interactions of their components (Carvacrol, thymol, eugenol, vanillin and etc.) with cell wall synthesis, which could affect fungal growth and its morphology (Rasooli *et al.*, 2006; Rao *et al.*, 2010). Some researchers have stated that some phenolic compounds present in the EOs could affect the plasma membrane and the cellular organelles, such as mitochondria of the fungi by decreasing the lipid and saturated fatty acid levels and increasing the unsaturated fatty acids, resulting in the leakage of Ca^{2+}, Mg^{2+} and K^{+} (Sharma and Tripathi, 2008). In addition, the existence of the hydroxyl groups and the aromatic nucleus could be a other important factor for the EOs antimicrobial activity (Numpaque *et al.*, 2011).

Fruit decay suppression

As shown in figure 2, the decay percentage of fruits increased with the storage time, variable from 1 to 3 weeks of incubation. The percentage of decay significantly decreased ($P<0.05$) with increasing of the concentration of *T. vulgaris* and *A. persica* EOs after 3 weeks of storage. After one week, no significant differences were observed between control and treated fruits (data not shown). After two weeks, significant differences were found among treatments with *R. officinalis* EO at 500 µl/l and *T. vulgaris* at 1000 µl/l. At the end of experiment, after two weeks, the maximum level of decay was related to the control fruits (70%), and the minimum one was attributed to *R. officinalis* (500 µl/l) and *A. persica* (1000 µl/l) EOs, reaching 12% and 13.3% decay, respectively.

These data agree with those obtained by Ramezanian *et al.* (2016) who showed the possibility of using *Z. multiflora* and *T. vulgaris* EOs to control postharvest citrus *Alternaria* decay (black rot). In addition, Elshafie *et al.* (2015) reported that *O. vul-*

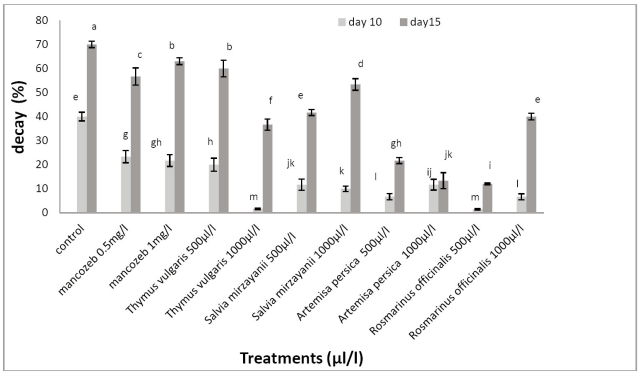

Fig. 2 - Suppressive effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on mangos decay after 10 and 15 days of storage compared to 'mancozed' (0.5 and 1.0 mg/l). Bars indicate the SD of the mean. Different letters indicate significant differences in mean values ($p<0.05$).

gare EO can control the brown rot of peach. Jhalegar *et al.* (2015) addressed their study on the influence of lemon grass, eucalyptus, clove and neem EOs against *P. digitatum* and *P. italicum* in 'Kinnow' mandarin. These authors showed that the decay rot during storage was less in the treated fruits than in the control ones. Duamkhanmanee (2008) reported that 4000 ppm lemon grass EO could control anthracnose by *C. gloeosporioides* decay of mangos. Phenolic compounds such as Carvacrol and thymol (Rao *et al.*, 2010) contained in EOs have a lipophilic molecular structure, therefore it interfer with membrane-catalyzed enzymes and cell wall, causing the cell death of microbes (Shirzad *et al.*, 2011). Many researchers believe that the type and the amount of phenolic compounds present in the oil can determine the antifungal activity of the EOs (Tripathi and Dukey, 2004).

Weight loss

The weight loss of fruit was increased strongly during the early weeks, but this increase was gradual throughout the storage period (Fig. 3). After two weeks of storage, the highest and lowest weight loss was observed in the control samples and those treated with 1000 µl/l *S. mirzayanii* (12.7% and 9.8% respectively). During the storage, the main mango weight loss (13.2%) was found in the control fruits, while the lowest one (10.8%) was observed in *R. officinalis* treated fruits at 500 µl/l concentrations.

The mechanism of EOs for reducing physiological

loss in weight might be related to the reduction of ethylene production and the respiration rate. Also, EOs cover the peel of fruit, creating the water barrier between the fruit and the environment, thereby reducing water exchange (Morillon *et al.*, 2002). This agrees with previous studies showing the efficacy of EOs in reducing the weight loss of cherries and grapes (Serrano *et al.*, 2005). Similarly, Du Plooy *et al.* (2009) reported that the use of *Mentha spicata* and *Lippia scaberrima* EOs reduced weight loss in 'Valencia' oranges.

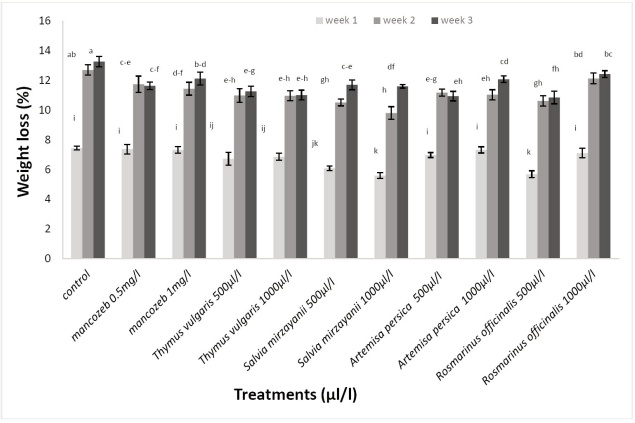

Fig. 3 - Effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on mangos weight loss after 1, 2, and 3 weeks of storage compared to 'mancozed' (0.5 and 1.0 mg/l). Bars indicate the SD of the mean. Different letters indicate significant differences in mean values ($p < 0.05$).

Fruit firmness

A continuous decline in mangos firmness was observed throughout storage (Fig. 4). However, the fruits treated with EOs showed higher firmness than the control ones. In each stage of storage, no significant difference was identified in the firmness of fruits, at different concentrations of EOs. After 3 weeks of storage, the firmness of control fruits was around 3.06 kg/cm^2, while the treated fruits were significantly firmer ($P<0.05$). In this stage, fruits treated with mancozeb showed no significant difference, as compared with those treated with 500 µl/l *T. vulgaris* EO. However, *R. officinalis* in both concentrations showed the highest firmness, as compared with other treatments.

Firmness, as one of the fruits properties, is a complex sensory attribute that also includes crispiness and juiciness; it is important in determining the acceptability of horticultural crops. It has been accepted that the loss of fruit firmness throughout the storage is mainly due to the depolymerization of cell wall components. Breakdown and the enzymatic degradation of insoluble protopectins into more simple soluble pectin can be associated with softening (Willats *et al.*, 2001). Ramezanian *et al.* (2016) found that the EOs reduced the activity of polygalacturonase and galactosidase, which are softening enzymes in the cell wall components, and maintained orange fruit firmness through the storage. The results obtained in the present study agree with those of Maqbool and Alderson (2010), who showed that by the application of lemongrass oil (0.05%) and Cinnamon oil (0.4%), the firmness of banana and papaya fruits was maintained during storage. However, Tzortzakis (2007) reported that eucalyptus and cinnamon EOs had no effect on the tomato and strawberry firmness.

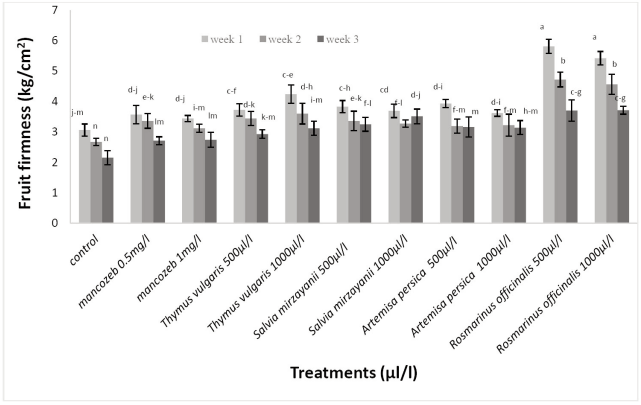

Fig. 4 - Effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on mangos firmness after 1, 2, and 3 weeks of incubation compared to 'mancozed' (0.5 and 1.0 mg/l). Bars indicate the SD of the mean. Different letters indicate significant differences in mean values ($p < 0.05$).

Surface color change

The results related to the changes in the fruit color (in terms of L*, a* and b*) of the treated mango showed that the lightness of the fruits peel was decreased throughout the storage time (Table 1). The fruits treated with 1000 µl/l *R. officinalis* EO retained higher L* over other treatments and control samples, after three weeks of storage. The highest and lowest L* was found in *R. officinalis* and *A. persica* at 1000 µl/l concentration, respectively. The results showed that a* was significantly decreased during the storage. However, the fruits treated with EOs maintained a higher a* than did the control ones. At the end of the storage, the lowest a* (7.46) and the highest a*(11.73) were found in the control and *R. officinalis* treated fruits at 1000 µl/l concentra-

tion, respectively. The results also showed that b* was increased during the storage time, but the trend in fruits treated by EOs was slower than that in the control. At the end of storage, *S. mirzayanii* and *R. officinalis,* at 500 µl/l concentrations, showed the minimum b* value (40.7 and 40.6), respectively. However, control and mancozeb samples showed the maximum b* value (56.6 and 55.9), respectively. The results obtained in the present study showed that EOs treatment could have better litheness with lower a* and b*, as compared to mancozed and control groups.

Ramezanian *et al*. (2016) showed the effect of the *Zataria multiflora* and *T. vulgaris* EOs on the black rot of 'Washington Navel' orange fruit. They found that the best color was related to zataria at 300 µl/l and thyme EOs at 400 µl/l concentrations. In agreement with our findings, Marjanlo *et al.* (2009) showed the effect of Cumin EO on the postharvest quality of strawberries, finding that the essential oil treated fruits maintained a higher L* during storage in comparison with the controls.

AA content

Ascorbic acid (vitamin C) content was gradually decreased during storage; however, its strength was lower in the treated samples (Table 2). Different concentrations of the EOs significantly maintained ascorbic acid content, as compared to the control. Overall, the most (14 mg/100 g/1) and the least (9 mg/100

Table 1 - Effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on mangos color change ± SD after 1, 2, and 3 weeks of storage compared to 'mancozed' (0.5 and 1.0 mg/l)

Testing index	Treatment (concentration)	Storage time		
		Week 1	Week 2	Week 3
L*	Control	63.3±0.43 a	47.5±0.56 n	46.2±0.56 n
	Mancozeb 0.5 mg/l	64.9±0.5 a	59.8±0.48 c-g	54.1±0.59 j-m
	Mancozeb 1 mg/l	55.3±.23 f-l	55.9±0.75 f-l	56.7±0.76 f-l
	Thymus vulgaris 500 µl/l	62.1±0.33 a-d	54.8±0.46 i-m	51.4±0.36 mn
	Thymus vulgaris 1000 µl/l	60.6±0.54 b-f	57.8±0.49 e-j	51.8±0.66 mn
	Salvia mirzayanii 500 µl/l	54.6±0.32 i-m	51.9±0.58 l-n	56.1±0.54 f-k
	Salvia mirzayanii 1000 µl/l	59.9±0.33 c-g	58.4±0.44 d-g	49.7±0.38 mn
	Artemisa persica 500 µl/l	59.7±0.43 c-g	56.9±0.58 f-l	48.7±0.65 n
	Artemisa persica 1000 µl/l	57.9±0.23 e-j	58.8±0.75 d-g	41.4±0.65 o
	Rosmarinus officinalis 500 µl/l	59.8±0.19 c-g	60.7±0.66 a-d	54.1±0.39 j-m
	Rosmarinus officinalis 1000 µl/l	62.2±0.25 a-d	65.3±0.76 a	59.5±0.53 c-g
a*	Control	16.4±0.87	12.6±0.87 g-i	7.4±0.87 l
	Mancozeb 0.5 mg/l	17.2±0.87	15.8±0.88 a-e	8.1±0.98 l
	Mancozeb 1 mg/l	16.8±0.99	16.1±0.98 a-d	8.6±0.99 kl
	Thymus vulgaris 500 µl/l	16.4±0.67	16.1±0.76 b-f	8.6±0.76 kl
	Thymus vulgaris 1000 µl/l	17.2±0.89	15.3±0.87 a-c	11.2±0.85 ij
	Salvia mirzayanii 500 µl/l	17.1±0.78	16.7±0.88 c-f	11.1±0.76 ij
	Salvia mirzayanii 1000 µl/l	17.6±0.77	15±0.68 e-g	8.4±0.87 kl
	Artemisa persica 500 µl/l	17.1±0.77	14.2±0.87 f-g	10.8±0.97 ij
	Artemisa persica 1000 µl/l	17.4±0.69	13.5±0.88 f-h	11.6±0.97 h-j
	Rosmarinus officinalis 500 µl/l	16.8±0.90	15.8±0.98 a-e	11.7±0.98 ij
	Rosmarinus officinalis 1000 µl/l	14.6±1.02	16.3±0.96 a-d	10.1±1.03 jk
b*	Cntrol	25.5±0.78 ij	34.7±1.02 e	56.6±2.2 a
	Mancozeb 0.5 mg/l	20.7±1.03 l-n	30±1.03 fg	55.9±3.2 a
	Mancozeb 1 mg/l	21.5±0.85 l-n	32.9±1.07 ef	46.7±2.9 bc
	Thymus vulgaris 500 µl/l	20.8±0.87 mn	25.1±0.84 h-j	46.7±3.8 bc
	Thymus vulgaris 1000 µl/l	20.6±0.78 mn	30.6±1.06 fg	49.4±3.5 b
	Salvia mirzayanii 500 µl/l	17.0±0.78 ab	30.7±0.94 fg	40.6±2.6 d
	Salvia mirzayanii 1000 µl/l	23.8±1.03 i-l	27.3±0.99 gh	48.3±3.5 bc
	Artemisa persica 500 µl/l	19.4±0.86 n	26.6±0.95 hi	47.8±3.6 bc
	Artemisa persica 1000 µl/l	20.8±0.98 l-n	30.8±1.04 fg	47.6±3.2 bc
	Rosmarinus officinalis 500 µl/l	20.7±1.03 l-n	24.4±0.90 i-k	40.7±2.8 d
	Rosmarinus officinalis 1000 µl/l	22.8±0.99 j-m	23.4±1.05 i-l	45.5±3.5 c

In each character, different letters indicate significant differences in mean values ($p<0.05$).

g/1) amount of ascorbic acid content was detected in the fruits treated with *S. mirzayanii* at the concentration of 500 µl/l and control after three weeks of storage, respectively. In general, fruits treated with mancozeb showed lower ascorbic acid content in comparison with those treated with EOs throughout the storage.

In agreement with our findings, Geransayeh *et al.* (2012) showed that the vitamin C content of grapes was decreased significantly during the storage; however, a higher vitamin C amount was observed in the samples treated with *T. vulgaris* EO. Our results were nevertheless in contrast with those of Marjanlo (2009) who did not detect any significant difference in the amount of ascorbic acid in strawberry fruits treated by the Essential Oils.

Carotenoids and total chlorophyll content

Analysis of the variance of carotenoids content revealed a significant difference ($p< 0.05$) between treatments (Table 2). The concentration of carotenoids content was low at the initial time of storage and then significantly increased during storage. At the end of the process, control fruits showed the highest content of carotenoids (1.94 mg 100 g^{-1}). *R. officinalis* and *T. vulgaris*, tested at 500 µl/l showing 1.09 and 1.15 (mg 100 g^{-1}) as carotenoids content, respectively, were the lowest one, as compared with other treatments. The total chlorophyll content was gradually decreased to a lower concentration in all

Table 2 - Effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on the ascorbic acid, carotenoids and total chlorophyll content ± SD in mangos after 1, 2, and 3 weeks of storage compared to 'mancozed' (0.5 and 1.0 mg/l)

Active metabolite	Treatment (concentration)	Storage time (week)		
		Week 1	Week 2	Week 3
Ascorbic acid	Control	12.3±3 cd	11±2 cd	9.5±4 d
	Mancozeb 0.5 mg/l	13.3±4 c	12.6±3 cd	11±3 cd
	Mancozeb 1 mg/l	13.5±4 c	12.3±3 cd	11±2 cd
	Thymus vulgaris 500 µl/l	14.8±7 bc	13.6±4 c	12±4 cd
	Thymus vulgaris 1000 µl/l	14.5±4 bc	13.7±4 c	12±5 cd
	Salvia mirzayanii 500 µl/l	17.4±4 a	16.4±4 b	14±5 bc
	Salvia mirzayanii 1000 µl/l	13.3±6 c	12.9±3 cd	12±4 cd
	Artemisa persica 500 µl/l	15.9±7 bc	14.8±2 bc	12±5 cd
	Artemisa persica 1000 µl/l	13.4±4 c	12.6±5 cd	11±5 cd
	Rosmarinus officinalis 500µl/l	17.5±4 a	17±5 b	13±3 c
	Rosmarinus officinalis 1000 µl/l	15.8±3 bc	14.5±4 bc	11±c 2 d
Carotenoids	Control	0.85±0.03kn	1.64±0.06 b	1.94±0.03 a
	Mancozeb 0.5 mg/l	0.69±0.04 m-o	1.57±0.03 b	1.43±0.03 b-e
	Mancozeb 1 mg/l	0.74±0.03 m-o	1.52±0.04 bc	1.48±0.08 b-d
	Thymus vulgaris 500 µl/l	0.74±0.07 m-o	1.12±0.05 g-j	1.15±0.03 f-i
	Thymus vulgaris 1000 µl/l	0.55±0.03 o	1.28±0.08 c-g	1.91±0.06 a
	Salvia mirzayanii 500 µl/l	0.69±0.08 m-o	1.26±0.05 d-h	1.39±0.07 b-f
	Salvia mirzayanii 1000 µl/l	0.7±0.06 m-o	1.1±0.06 g-k	1.53±0.03 bc
	Artemisa persica 500 µl/l	0.56±0.05 o	0.85±0.04 l-n	1.31±0.04 c-g
	Artemisa persica 1000 µl/l	0.6±0.06 o	0.87±0.08 j-m	1.22±0.03 e-h
	Rosmarinus officinalis 500 µl/l	0.52±0.04 o	0.93±0.05 i-m	1.09±0.04 g-l
	Rosmarinus officinalis 1000 µl/l	0.5±0.07 o	1.01±0.03 h-l	1.16±0.05 f-i
Total chlorophyll	Control	2.72±0.2 f	1.19±0.02 gh	0.03±0.002 i
	Mancozeb 0.5 mg/l	3.98±0.4 e	1.31±0.02 g	0.53±0.03 h
	Mancozeb 1 mg/l	3.44±0.2 f	1.72±0.02 g	0.31±0.02 h
	Thymus vulgaris 500 µl/l	4.86±0.5 de	2.5±0. 6 f	0.11±0.01 h
	Thymus vulgaris 1000 µl/l	4.33±0.2 de	2.66±0. 5 f	0.11±0.02 h
	Salvia mirzayanii 500 µl/l	5.43±0.3 d	3.55±0.2 f	0.16±0.01 h
	Salvia mirzayanii 1000 µl/l	7.62±0.2 bc	3.47±0.2 f	0.15±0.02 h
	Artemisa persica 500 µl/l	9.61±0.2 a	2.58±0.7 f	0.16±0.03 h
	Artemisa persica 1000 µl/l	8.38±0.3 b	2.35±0.5 f	0.22±0.02 h
	Rosmarinus officinalis 500 µl/l	8.69±0.2 ab	3.27±0.8 f	0.16±0.04 h
	Rosmarinus officinalis 1000 µl/l	8.19±0.4 b	3.3±0.6 f	0.32±0.5 h

In each character, different letters indicate significant differences in mean values ($p<0.05$).

treatments throughout the storage (Table 2); however, the highest reduction was observed in the control fruits. At the end of storage, the minimum chlorophyll content (0.03) was recorded in the control, while the highest was found in mancozeb with 0.5 mg/l concentration.

TSS, TA and pH

A gradual increase in TSS percentages was determined in all treatments (Table 3). Generally, the fruits treated with EOs had lower TSS percentages than the control fruits throughout the storage. However, the treated fruits did not show any significant difference in TSS, as compared with the controls. These results were in agreement with those in other studies (Marjanlo *et al.*, 2009). Nevertheless, they are in discordance with Rabiei *et al.* (2011), who reported that thyme EOs treatment had a significant effect on the pH of apples. As shown in Table 3, TA values were also gradually decreased during storage. At the end of storage, the maximum TA values (0.27%) were observed in *A. persica* (1000 µl/l), while the minimum (0.10%) was in the control fruits. Among the EOs treatments, *R. officinalis* (1000 µl/l) resulted in the lowest acidity (0.17%), this was followed by *T. vulgaris* (0.16%) with 1000 µl/l concentration during storage; however, no significant difference was observed between the treatments. These results are in agreement with those reported by Maqbool and Alderson (2010), who showed that the

Table 3 - Effect of the *Thymus vulgaris*, *Salvia mirzayanii*, *Artemisia persica* and *Rosmarinus officinalis* EOs tested at 500 and 1000 µl/l on Total Soluble Solids (TSS), pH, and Titratable Acidity (TA) ± SD in mangos after 1, 2, and 3 weeks of incubation compared to 'mancozed' (0.5 and 1.0 mg/l)

Quality parameter	Treatment	Storage time (week)		
		Week 1	Week 2	Week 3
TSS	Control	8.9±3 c	9.6±3 b	9.9±2 a
	Mancozeb 0.5 mg/l	8.06±3 cd	9.23±5 b	9.5±3 b
	Mancozeb 1 mg/l	8.33±4 c	9.13±5 bc	9.5±2 b
	Thymus vulgaris 500 µl/l	8.96±5 c	9.63±4 b	9.7±2 ab
	Thymus vulgaris 1000 µl/l	8.83±4 c	9.76±4 ab	9.8±4 ab
	Salvia mirzayanii 500 µl/l	8.7±4 c	9.26±2 b	9.3±3 b
	Salvia mirzayanii 1000 µl/l	8.33±3 c	9.26±5 b	9.6±2 b
	Artemisa persica 500 µl/l	8.66±3 c	9.3±3 b	9.6±3 b
	Artemisa persica 1000 µl/l	8.1±5 cd	9.53±5 b	9.7±4 b
	Rosmarinus officinalis 500 µl/l	7.5±3 d	9±4 bc	9.2±2 b
	Rosmarinus officinalis 1000 µl/l	8.66±4 c	9.53±5 b	9.5±3 b
pH	Control	1.15±0.03 cd	2.89±0.2 c	4.75±1.4 a
	Mancozeb 0.5 mg/l	1.06±0.02 cd	2.38±0.4 c	4.3± 2 ab
	Mancozeb 1 mg/l	1.09±0.02 cd	2.59±0.3 c	4.3± 2 ab
	Thymus vulgaris 500 µl/l	1.06±0.02 cd	2.51±0.2 c	4.18± 2. 2 ab
	Thymus vulgaris 1000 µl/l	1.26±0.02 cd	2.54±0.3 c	4.31±1.8 ab
	Salvia mirzayanii 500 µl/l	0.99±0.02 d	2.74±0.2 c	4.24±1.9 ab
	Salvia mirzayanii 1000 µl/l	1.12±0.02 cd	2.26±0.2 c	4.42±2 ab
	Artemisa persica 500 µl/l	1.03±0.02 cd	2.45±0.3 c	4.48±3 ab
	Artemisa persica 1000 µl/l	1.08±0.02 cd	2.17±0.2 c	4.47±1.4 ab
	Rosmarinus officinalis 500 µl/l	1.02±0.02 cd	1.76±0.1 cd	4.36±1.2 ab
	Rosmarinus officinalis 1000 µl/l	1.07±0.02 cd	2.52±0. 5 c	4.56±2.1 ab
TA	Control	0.208±0.03 c	0.196±0.01 c	0.10±0.009 d
	Mancozeb 0.5 mg/l	0.254±0.02 bc	0.255±0.01 bc	0.23±0.02 bc
	Mancozeb 1 mg/l	0.27±0.02 bc	0.234±0.02 bc	0.23±0.02 bc
	Thymus vulgaris 500 µl/l	0.328±0.02 b	0.26±0.03 bc	0.23±0.02 bc
	Thymus vulgaris 1000 µl/l	0.308±0.01 b	0.262±0.02 bc	0.16±0.01 cd
	Salvia mirzayanii 500 µl/l	0.384±0.02 b	0.267±0.03 bc	0.25±0.02 bc
	Salvia mirzayanii 1000 µl/l	0.352±0.03 b	0.299±0.03 bc	0.24±0.02 bc
	Artemisa persica 500 µl/l	0.288±0.02 bc	0.277±0.02 bc	0.23±0.02 bc
	Artemisa persica 1000 µl/l	0.405±0.02 b	0.307±0.01 b	0.27±0.02 bc
	Rosmarinus officinalis 500 µl/l	0.471±0.02 a	0.302±0.02b	0.26±0.02 bc
	Rosmarinus officinalis 1000 µl/l	0.328±0.01 b	0.261±0.01 bc	0.17±0.01 cd

In each character, different letters indicate significant differences in mean values ($p<0.05$).

maximum reduction of the TA values was observed in the control fruits of bananas and papayas. Data related to the changes in the pH of fruits during storage revealed a significant increase in all treatments (Table 3). In each time, a higher pH value was found in the control groups. These results were in line with those reported by Du Plooy *et al*. (2009) and Tzortzakis (2007), showing no significant differences between the pH of control and treated fruits

4. Conclusions

The present study proves that the *T. vulgaris*, *A. persica*, *R. officinalis* and *S. mirzayanii* EOs could be employed under postharvest condition to control a pathogenic isolate of *A. niger* on mango fruits. The effectiveness of these EOs was more than mancozeb. So that, the EOs here tested could be used as a natural fungicide to control an isolate of *A. niger* during postharvest mangos. However, further studies are needed to fully understand the antimicrobial mechanisms incited by these EOs on a wide range of *A. niger* isolates, and evaluate their commercial implementation in order to increase the storage lifetime and quality of this marketable commodity.

Acknowledgements

This study as research project was financially supported by Hormozgan University of Medical Sciences, Bandar Abbas, Iran. Authors are thankful for providing necessary facilities for carrying out this work.

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